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Semisynthesis of an Analogue of Antitumor Bicyclic Hexapeptide RA-VII by Fixing the Ala-2/Tyr-3 Bond to *Cis* by Incorporating a Triazole *cis*-Amide Bond Surrogate

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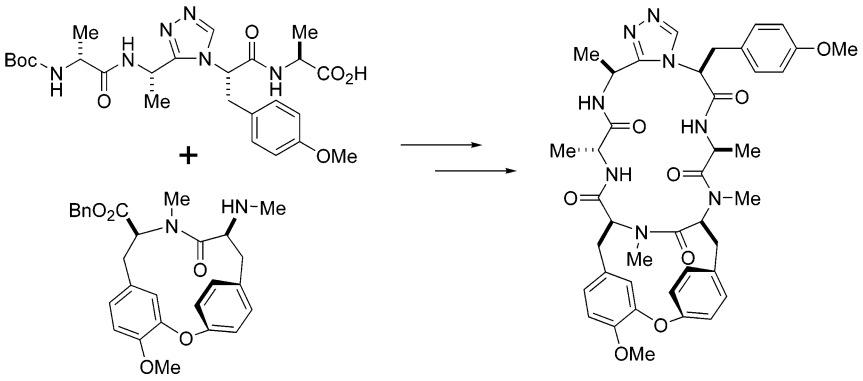
ABSTRACT



We prepared an analogue of an antitumor bicyclic hexapeptide RA-VII whose amide configuration between residues 2 and 3 was fixed to *cis* by incorporating a triazole *cis*-amide bond surrogate. This analogue was shown, by NMR studies, to take almost the same conformation as that of the minor conformer of RA-VII. It showed no cytotoxic activity.

RA-VII (**1**)[1,2] and bouvardin (**2**)[3] are plant-origin antitumor bicyclic hexapeptides having a unique 14-membered cyclo-isodityrosine unit. Their antitumor action is considered to be due to inhibition of protein synthesis through interaction with eukaryotic 80 S ribosomes,[4,5] and accordingly, for effective interaction a particular molecular conformation is considered to be essential. In solution, due to the *cis*/*trans* isomerization at the three tertiary amides, cyclic peptides of this series can take several conformations. Peptide **1** takes two, and sometimes three, stable conformations. Their ratios vary, but of those three conformers, one conformer in which the amide bond between Ala-2/Tyr-3 is *trans* (major) and another one in which it is *cis* (minor) are always observed in solution (Figure 1). The third minor one observed occasionally involves isomerization at the amide bonds between Ala-2/Tyr-3 and between Ala-4/Tyr-5. The amide

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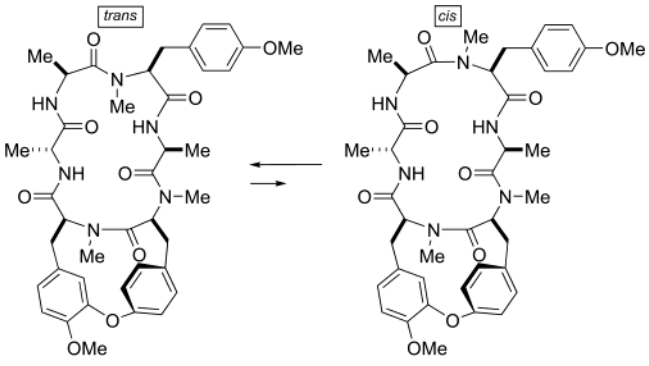
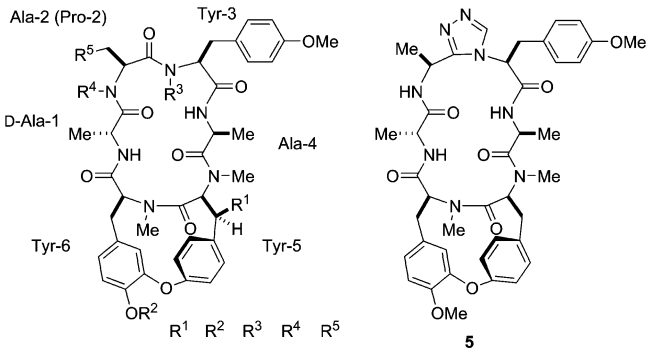


solvent	population	
	t-t-t-t-c-t	*t-c-t-t-c-t*
$CDCl_3$	89%	11%
CD_3OD	84%	16%

Figure 1. Population of the two conformers of **1** in solution.

configuration in the major conformer is thus *trans-trans-trans-trans-cis-trans* (*t-t-t-t-c-t*) whereas that of the minor conformer is *t-c-t-t-c-t* at D-Ala-1/Ala-2, Ala-2/Tyr-3, Tyr-3/Ala-4, Ala-4/Tyr-5, Tyr-5/Tyr-6 and Tyr-6/D-Ala-1, respectively, which has been verified by X-ray crystallography, NOESY experiments, and computational methods.[6−8] The major conformer has been identified as an active conformer on the grounds that [Pro-2]RA-VII (**3**)[9] and [*N*-desmethyl-Tyr-3]RA-VII (**4**),[10,11] both taking a single conformer of *t-t-t-t-c-t* amide configuration in solution, expressed a significant cytotoxic activity. However, it is not known whether the minor conformer characterized by having a *cis* amide bond between Ala-2/Tyr-3 or *t-c-t-t-c-t* amide configuration shows any activity or not. In the present study, we designed and prepared an analogue of RA-VII whose conformer in solution should be exclusively of the *t-c-t-t-c-t* amide configuration and examined its solution conformation and cytotoxicity.

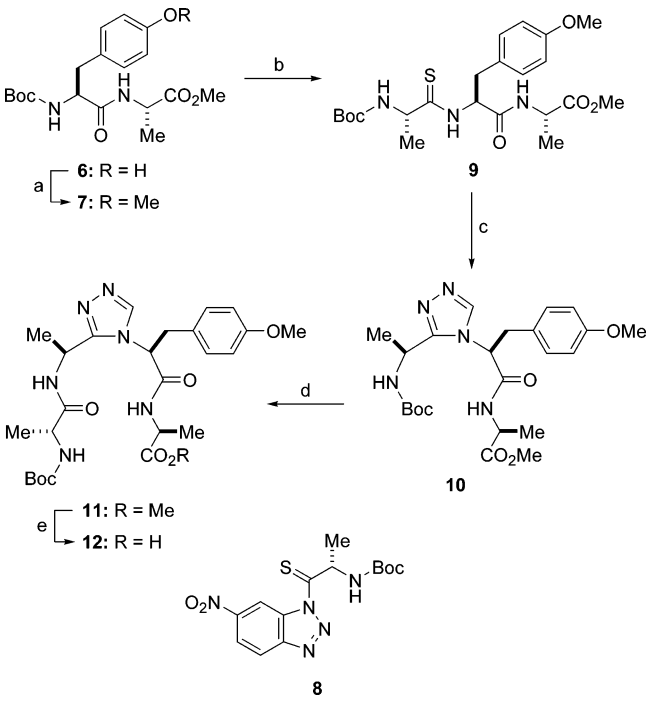


	R^1	R^2	R^3	R^4	R^5
RA-VII (**1**)	H	Me	Me	H	H
bouvardin (**2**)	OH	H	Me	H	H
[Pro-2]RA-VII (**3**)	H	Me	Me	CH_2-CH_2	
4	H	Me	H	H	H

5

The major feature of our present preparation of a peptide **1** analogue having *t-c-t-t-c-t* amide configuration (**5**) included fixing the amide bond between Ala-2 and Tyr-3 residues to *cis* by replacing the relevant amide bond with a triazole *cis*-amide bond surrogate, because the configuration at the other two tertiary amides, between Ala-4/Tyr-5 and between Tyr-5/Tyr-6, are less flexible and, apparently, usually are *trans* and *cis*, respectively, in solutions. This approach of fixing an amide bond by incorporating this surrogate has an advantage that the desired product is prepared by reacting readily available chiral thionopeptides with the reagents under mild conditions without affecting the side chains and other chiral centers.[12] Thus, by a sequence of reactions as shown in Scheme 1, first, the tetrapeptide fragment **12**, incorporating

Scheme 1[a]



[a] Reagents and conditions: (a) NaH, MeI, DMF, 82%; (b) 4 M HCl−dioxane; **8**, Et_3N, THF, 87%; (c) $H_2NNHCHO$, $Hg(OAc)_2$, MeCN; *p*-TsOH, powdered 4 Å MS, $CHCl_3$, 79%; (d) 4 M HCl−dioxane; Boc-D-Ala-OH, EDC, HOBt, Et_3N, $CHCl_3$, 89%; (e) LiOH, THF−MeOH−H_2O, 95%.

a *cis*-amide bond surrogate (residues 1−4), was prepared, which was linked to the cycloisodityrosine unit (residues 5 and 6) **13** and then subjected to macrocyclization (Scheme 2). For macrocyclization, a critical step in the synthesis of cyclopeptides, the carboxyl group of Tyr-6 and the amino

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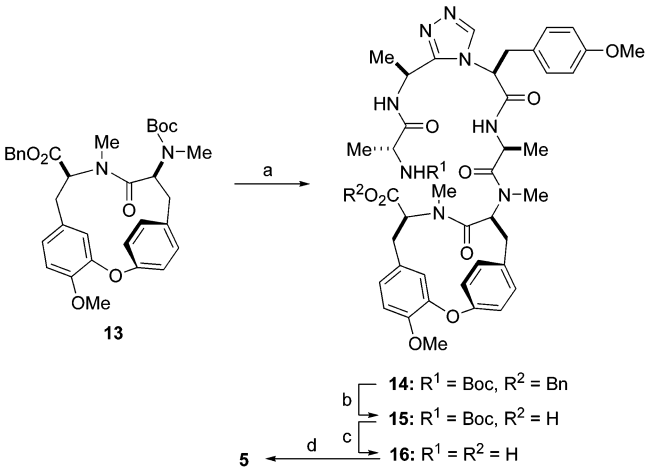
Scheme 2[a]



[a] Reagents and conditions: (a) TFA; **12**, EDC, HOObt, THF, 73%; (b) H_2, Pd/C, EtOH, 94%; (c) 4 M HCl−dioxane; (d) FDPP, *N*,*N*-diisopropylethylamine, DMF, 9.5% from **15**.

group of D-Ala-1 were chosen as the cyclization site, as was the case for the RA-VII syntheses.[13]

Preparation of tetrapeptide fragment **12** proceeded as shown in Scheme 1, starting with O-methylation of the N-protected tyrosyl-alanine methyl ester **6** to afford methyl ether **7** in 82% yield. After removal of the Boc group of **7**, the resultant amine was reacted with benzotriazole thioacylating agent **8**[14] to provide thionotripeptide **9** in 87% yield. Treatment of **9** with formic hydrazide and mercury(II) acetate and subsequent dehydration using *p*-toluenesulfonic acid and powdered 4 Å molecular sieves afforded triazolotripeptide **10** in 79% yield. Tripeptide **10** was converted into tetrapeptide **11** by the standard procedure (89%), which, after the subsequent hydrolysis of the methyl ester group, afforded carboxylic acid **12** in 95% yield (Scheme 1).

After removal of the Boc group, cycloisodityrosine **13**, prepared by partial degradation of natural RA-VII (**1**),[15] was coupled to acid **12** to provide hexapeptide **14** in 73% yield. Debenzylation of **14** afforded **15** in 94% yield, and subsequent removal of the *N*-Boc group by treatment of **15** with 4 M HCl in dioxane afforded free hexapeptide **16**. Then, **16** was subjected to macrocyclization by treating it with pentafluorophenyl diphenylphosphinate (FDPP, 4 equiv) and *N*,*N*-diisopropylethylamine (6 equiv) in DMF (concentration 0.002 M, 0 °C, 3 d, then room temperature, 2 d) to link the Tyr-6 and D-Ala-1 residues together to afford cyclopeptide **5** (9.5% yield from **15**). The yield of macrocyclization of **16** to **5** was very low: the incorporated *cis*-amide bond surrogate may interfere with **16** taking suitable conformations for macrocyclization. In another experiment using diphenylphosphoryl azide (DPPA, 4 equiv) and $NaHCO_3$ (8 equiv), this macrocyclization was even less efficient (yield 6.5%), though DPPA was reportedly successfully employed in the synthesis of RA-VII (**1**).[13b,c]

The solution conformation of analogue **5** was studied by NMR experiments. In CD_3OD, analogue **5** was shown to exist in a single conformation. The key NOESY correlations are shown in Figure 2. The correlation between Ala-2 H_α/Tyr-3 H_α indicated that the configuration of the peptide bond between Ala-2/Tyr-3 residues was exclusively *cis* as expected. The correlations between Ala-4 Me/Tyr-5 NMe and between Ala-4 H_α/Tyr-5 NMe indicated that the amide bond between Ala-4/Tyr-5 was *trans*, and the correlation between Tyr-5 H_α/Tyr-6 H_α indicated that the amide bond between Tyr-5/Tyr-6 was *cis*. Thus, analogue **5** was determined to take the same *t*-*c*-*t*-*t*-*c*-*t* amide configuration in solution as that of the minor conformer of peptide **1**. The chemical shift and coupling constant values of analogue **5** and the minor conformer of peptide **1**, having *t*-*c*-*t*-*t*-*c*-*t* amide configuration, were generally quite similar, indicating that their solution structures were approximately identical. In addition to those observations, a transannular NOESY correlation observed between D-Ala-1 H_α/Ala-4 Me further demonstrated that the structures of the backbone conformation of analogue **5** and the minor conformer of peptide **1** were almost identical.[7]

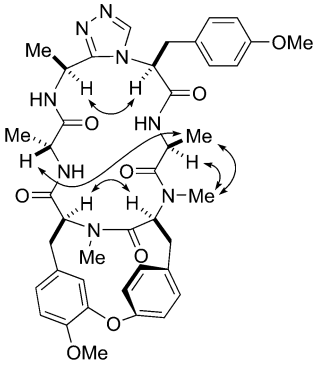


Figure 2. Key sequential and transannular NOESY correlations for **5** in CD_3OD.

Analogue **5** and peptide **1** were evaluated for the cytotoxic activity using P-388 murine leukemia cells. Their IC_{50} values were >10 and 0.0027 μg/mL, respectively. We may conclude, therefore, that the minor conformer of **1** having the *t*-*c*-*t*-*t*-*c*-*t* configuration, accompanying the major conformer in solution in all solvents tested, does not possess significant activity.

The present and the previous results,[9−11] demonstrating that the major conformer with the *t*-*t*-*t*-*t*-*c*-*t* configuration is responsible for the activity, whereas the minor one with the *t*-*c*-*t*-*t*-*c*-*t* configuration takes little, if any, part in expressing the activity, provide further knowledge about the conformation−activity relationships of the peptides of this series,

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regarding the mechanisms of interactions between the peptides and the ribosomes. The results also emphasize the importance of the conformation in the expression of biological activities and suggest that more active analogues or analogues having new functions such as resistance against enzymatic hydrolysis may be designed and synthesized, e.g., by replacement of the amide bonds of peptide **1**, having *t*-*t*-*t*-*c*-*t* amide configuration, with *cis*- or *trans*-amide bond isosteres.

Supporting Information Available: Experimental details and ^{1}H NMR spectra of **5**, **7**, **9**−**12**, **14**, and **15**. This material is available free of charge via the Internet at http://pubs.acs.org.

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